A LOW-COST, LABEL-FREE DNA DETECTION METHOD BASED ON DIRECT ELECTRONIC READ IN LAB-ON-CHIP FORMAT,WITH APPLICATION TO LONG-RANGE PCR

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ABSTRACT

Here we report a new label-free DNA detection method with direct electronic read, and apply it to long-range PCR. This method uses a nonlinear electrohydrodynamic instabilities phenomenon discovered in the nineties: when subjected to high electric fields (up to 320 V/cm at 15Hz), suspensions of large charged macromolecules in microchannel, such as long DNA molecules, create "giant" dynamic concentration fluctuations.These fluctuations are associated with large conductivity fluctuations, and we use here a contact-mode local conductivity detector to detect these fluctuations.

KEYWORDS

Label-free DNA,long range PCR,contact conductivity detection,microchip capillary electrophoresis, wavelet analysis.

INTRODUCTION

In order to evolve from a "chip in the lab" to a "lab on a chip" paradigm, there is still a strong demand for label-free, low-cost and portable detection technologies, notably for sequence-specific detectionat low concentrations. Conductivity measurements have the advantage of being universal, low-cost and compatible with direct electronic detection. However the high voltage required to create DNA aggregates[1] (up to 320 V/cm at 15 Hz) leads to a technological difficulty, already known in earlier works dedicated to conductimetric or impedancemetric detection in microchip capillary electrophoresis: the presence of electrical interactions between the high voltage power supply (HVPS) and the (typically low voltage)detection electronics [2]. In order to decouple the detection electronics from the high voltage excitation one, an original and simple "doubly symmetric" floating mode battery-operated detection scheme was developed. A wavelet analysis is then applied to unravel from the chaotic character of the electrohydrodynamic instabilities a scalar signal robustly reflecting the amplification of DNA.

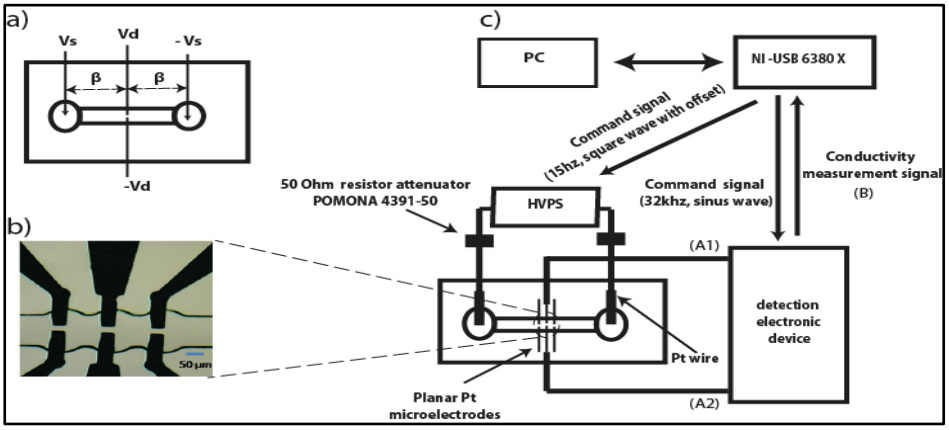

Figure 1: a)Themicrochannel is 5mm length, 40µm high and 150µm wide excepted in the detection zone in the middle of microchannel where the width is 160µm. Electrodes for conductivity measurement in contact (ECMC) consist in two planar and miniaturized Pt electrodes with 50µm width, 25µm gap and 200nm thick, positioned perpendicularly to the microchannel, facing each other, in the detection zone. Balanced voltages are supplied from a high voltage power supply(HVPS) to both side of microchannel(Vs ,-Vs), and balanced conductivity detection voltages (32Khz frequency) are applied on the ECMC (Vd, -Vd) ***b)****Three ECMC with associated detection zones. Only one is used for measurements.* ***c)*** *A schematic diagram of the measurement set-up.*

METHOD

Microchip fabrication. The microchip is a Glass/PDMS (Polydimethylsiloxane) hybrid microfluidic chipfabricated by rapid prototyping. It consists in two layers: a PDMS layer,which contains the microchannel, and a glass substrate, which supportselectrodes for conductivity measurement in contact (ECMC) fabricated by lift-off(figure 1a -1b).

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Detection electronics. We developed a system with floating ground based on 6 lithium batteries (ultralife, 9V).This system consists in 3 low-cost isolation amplifiers ISO124P, 2 voltage controlled current sources, 1 bridge of resistors and 1 differential amplifier to get an all-electronic, portable and simple device, with a high sensitivity. A schematic diagram of the balanced circuit is shown in Figure 2.

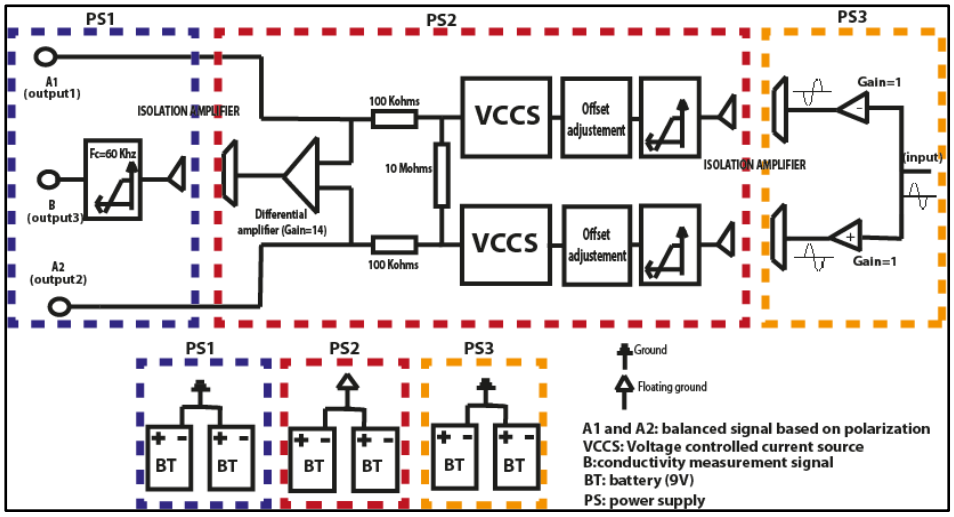

Figure2:A schematic diagram of the balanced circuits designed for the detection electronics. Isolation amplifiers eliminate the ground loop due to leakage currents. The low-pass filters are used to suppress ripple voltages from isolation amplifiers.

Experimental procedures. This work is the first implementation of a completely new technology for on-chip detection of DNA. It involves two innovations: the detection principle itself, and the nature of the sample detected in chip :long-range PCR products. We performed the PCR out of chip, checked the products by conventional gel electrophoresis, and injected in the microfluidic chip the raw product of the reaction.Figure 1C shows a schematic of the set-up.

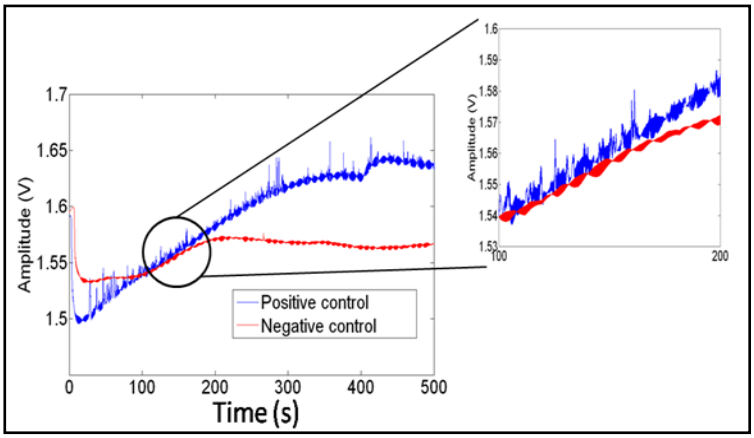

*Figure **3:** Example of conductivity signals based on 10Hz frequency demodulation of 32Khz conductivity measurement signal obtained from raw PCR solutions of negative (without DNA) and positive (with DNA target) control resulting to 38 cycles of amplification.*

Analysis. Figure3 shows an example of results of conductivity signals wherein there is a monotonous baseline drift, and a high frequency noise with an irregular roughly periodic pattern. Because the relevant part of the signal is not periodic and is chaotic in nature, the wavelet analysis is a qualitatively required component of the analysis. In the wavelet analysis, the input signal is convoluted with a set of functions (a wavelet basis) generated from an appropriate local functional pattern, that best matches the transient events of interest.

RESULT

We tested the ability of our wavelet analysis procedure to selectively extract DNA aggregates signals, by simultaneously recording the conductivity signal, fluorescent images and the local fluorescence signal in the gap between the ECMC (Figure4). Finally, our new technology performance was assessed by applying it to the quantification of long-

range PCR amplification products from different initial amount of DNA lambda (from 0 to 2.5ng) at different numbers of amplification cycles (from 0 to 41 cycles) (figure 5). The limit of detection (LOD) of the present label-free method is between 10 and 100fg of initial DNA, at least as good as fluorescence detection.

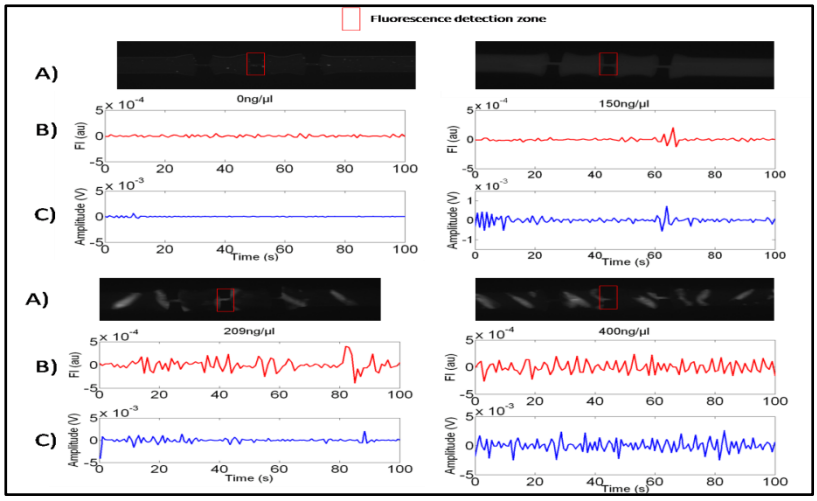

Figure 4: For increasing amounts of 10kbp DNAextracted from the PCR mixture , suspended in TE (1X) buffer and labeled with Syber Gold , correlations are shown between observations of DNA aggregates with A) fluorescence imaging, B) the time-derivative ratio of the fluorescence intensity measured in the detection zone to the one outside the microchannel (FI), C) the specific conductivity signal based on DNA aggregates extracted by the wavelet analysis procedure.

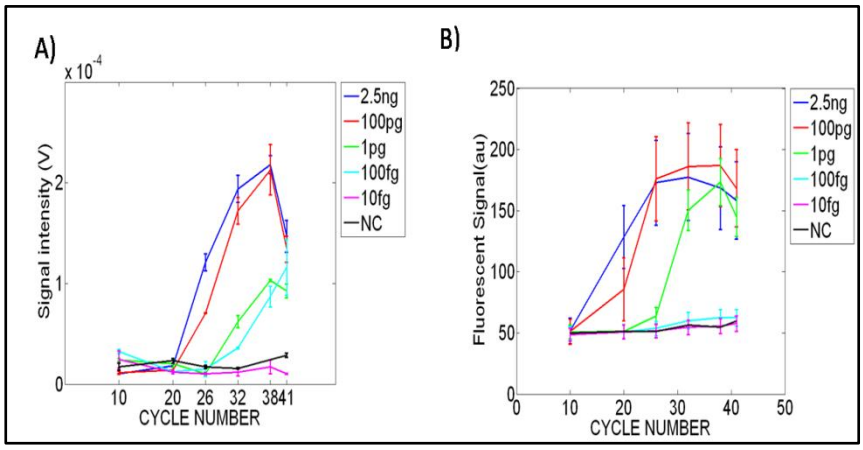

Figure 5: A)Label-free quantification of DNA using the wavelet analysis procedurefor various amount of target DNA (10fg, 100fg ,1pg, 100pg, 2.5ng) at various amplification cycles (10, 20, 26, 32, 38, 41). The standard deviation for each point in panel was calculated from 3 measurements from 3 different aliquots. B) Corresponding densitometric analysis of gels performed as control.

CONCLUSION

This new concept opens the route to low-cost portable devices for end-point DNA detection, with a sensitivity better than 100 fg/µl. The doubly symmetric contact conductivity detection might find many applications in label-free electrokinetic separations on chip. The wavelet pattern recognition approach could also help to solve various problems of pattern recognition in lab-on-chip applications.

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